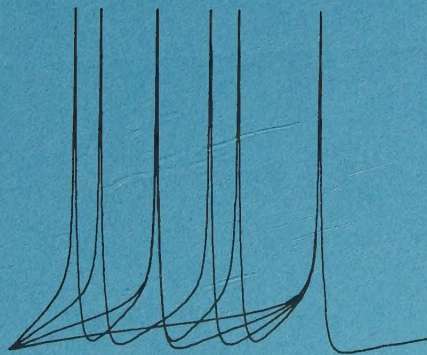


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Control of Impulse Firing in Lobster Stretch Receptor Neurones

By
Staffan Gestrel^us



Lund 1983

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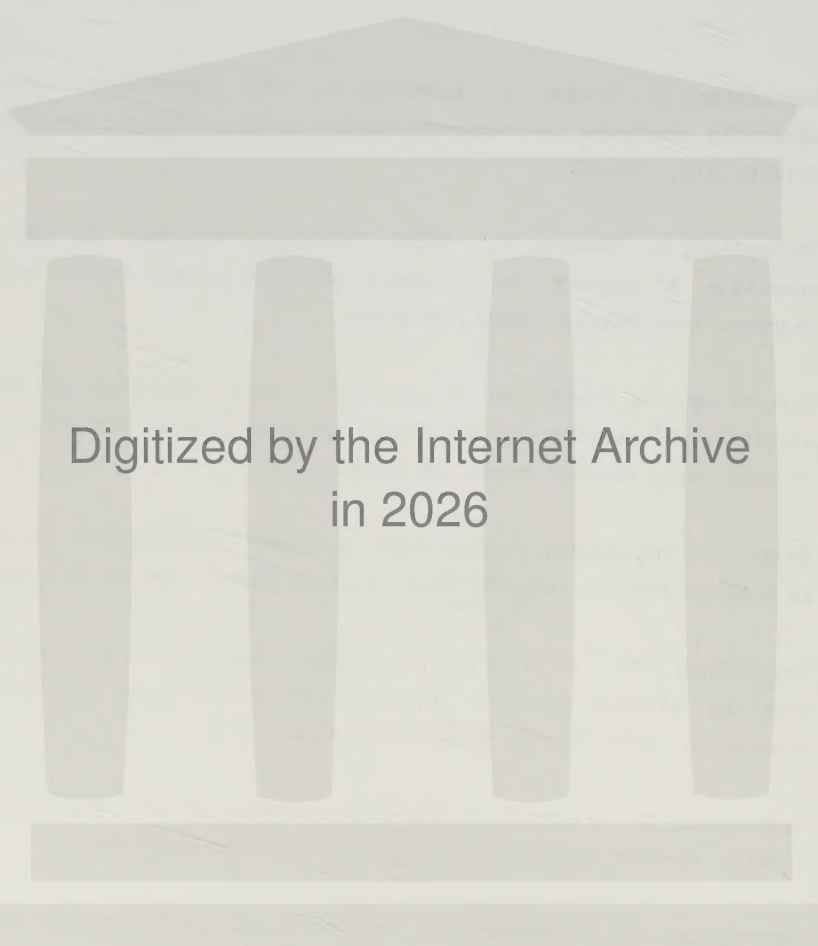
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This thesis is based on the following articles which in the text will be referred to by their Roman numerals.

- I. Gestrelus, S., Grampp, W. & Sjölin, L. 1981. Subthreshold and near-threshold membrane currents in lobster stretch receptor neurones. *J Physiol* 310, 191-203.
- II. Gestrelus, S., Grampp, W. 1983. Kinetics of the TEA and 4-AP sensitive K^+ current in the slowly adapting lobster stretch receptor neurone. *Acta Physiol Scand*. In press.
- III. Gestrelus, S., Grampp, W. & Sjölin, L. 1983. Kinetics of the TTX sensitive Na^+ current in the slowly adapting lobster stretch receptor neurone. *Acta Physiol Scand*. In press.
- IV. Edman, Å., Gestrelus, S. & Grampp, W. 1983. Intracellular ion control in lobster stretch receptor neurone. *Acta Physiol Scand*. In press.
- V. Gestrelus, S. & Grampp, W. 1983. Impulse firing in the slowly adapting stretch receptor neurone of lobster and its numerical simulation. *Acta Physiol Scand*. In press.
- VI. Gestrelus, S. & Grampp, W. Membrane currents in the rapidly adapting lobster stretch receptor neurone. Submitted for publication.
- VII. Gestrelus, S. & Grampp, W. Impulse firing in the rapidly adapting stretch receptor neurone of lobster and its numerical simulation. Submitted for publication.



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INTRODUCTION

An important property of nerve cells is their ability to translate external stimulation into impulse activity. This basic function is found both in regular neurones, where stimulation is often due to transmitter action, as well as in specialized receptor cells or neurones which are able to selectively react to various physical or chemical stimuli in their environment.

Operationally, the process of translation from stimulation to impulse firing can be subdivided into two main steps consisting of transformation of the external stimulus into generator current by a transducer mechanism, and a subsequent conversion of the generator current into impulse activity as result of a so-called encoder action (Borsellino et al. 1966, Chaplain et al. 1971). In many neurones and receptors the transducer and encoder functions are located within the same cell. In other structures, where this is not the case (eg. the receptor mechanism of the inner ear) the two main steps of information processing are normally linked by an additional step of synaptic transmission.

Although both transducer and encoder mechanisms have been the objects of a great number of investigations there are still many uncertainties regarding their nature and modes of operation. In this situation it seemed justified to start the present investigation which, primarily, aims at elucidating further details of the encoder function. In studies of this kind it is customary to replace the transducer function by application of stimulating current from an external and controllable source. In the following this kind of current application will be referred to as stimulus or stimulation of the preparation.

It is well known that as a result of specific encoder properties the firing output of an excitable cell may show both a stimulation and time dependence. The time dependence of a repetitive activity is often referred to as adaptation (Adrian & Zotterman 1926), while the stimulus dependence is expression of the cell's stimulus sensitivity which, in general terms, may be defined by the relation between stimulation intensity and resulting firing frequency.

In the many previous investigations trying to explain the underlying principles of adaptation and stimulus sensitivity it is now possible to discern two main lines of explorative techniques. According to one of these techniques the encoder is regarded as a "black box" whose characteristics can be properly described by a single input (stimulus) - output (firing frequency) relationship (Borsellino et al. 1966, Chaplain et al. 1971). In retrospect it may be concluded that this kind of scientific approach is rather unprofitable, since it implies linear systems analysis on non-linear systems with a discontinuous output which severely restricts both the applicability of the method and the interpretation of the results obtained (cf. Baker Jr & Hartline 1978). It is also true that, even if a transfer function is found which reasonably well predicts the system behaviour, it has little explanatory power, since the mathematical formulations inferred may be quite unrelated to the cellular mechanisms underlying firing control (cf. Fohlmeister et al. 1974, 1977a, b).

In an alternative approach attempts are made to explain the encoder function from measurements of various specific membrane properties. This method is based on the classical study of Hodgkin & Huxley (1952) in which, after identification and kinetic characterization of different ion currents, it was possible to develop a mathematically formulated theory of the action potential. In its original form the Hodgkin-Huxley model is, like many of its followers (Frankenhaeuser & Huxley 1964, Frankenhaeuser & Vallbo 1965, Connor & Stevens 1971a, b, c), unable to reproduce relatively slow time dependent changes like adaptation of the impulse activity. However, such phenomena could later be accounted for by minor modifications. Thus, by adding a slowly subsiding K^+ current to a somewhat altered Frankenhaeuser-Huxley model Kernell & Sjöholm (1972, 1973) were able to reproduce initial adaptation in spinal motoneurons. By assuming a similar type of K^+ current a model of initial adaptation has later also been developed for dorsal spinocerebellar tract neurones (Gustafsson 1974).

For adaptation processes lasting for seconds or longer various mechanisms have been suggested. These include activation of a hyperpolarizing pump current as in crayfish stretch receptor (Sokolove & Cooke 1971), activation of a slowly increasing K^+ current as in *Aplysia* giant axon (Brodwick & Junge 1972), or a combination of both of these mechanisms as in leech neurones (Jansen & Nicholls 1973). However, so far the only quantitative model of prolonged adaptation based on purposeful experimental analysis has

been presented by Partridge & Stevens (1976). Their formulations contain, as adapting mechanism, the description of a slowly developing K^+ current inferred from measurements on the marine molluscs *Archidoris monterreyensis* and *Anisodoris nobilis*. In addition to such investigations theoretical studies have also been performed in which pertinent data given in the literature were incorporated into mathematical models for the purpose of classifying (Colding-Jørgensen 1976) or explaining (Scriven 1981) various types of long-term firing modulation.

In the present investigation further attempts are made to clarify the encoder function by developing a mathematical theory based on measurements of various cell parameters. As basic hypothesis of the study it was presumed that the stimulation sensitivity and adaptation properties of the encoder are determined by a special balance of membrane currents in sub- and near-threshold voltage regions (cf. Eyzaguirre & Kuffler 1955). As experimental material were chosen slowly and rapidly adapting lobster stretch receptor neurones (Alexandrowicz 1951a, b). These cells are easy to keep isolated in a controllable environment. Also, in preliminary tests (Gestrelus & Grampp 1978, Gestrelus & Grampp 1980), they had been found to display slow changes in sub- and near-threshold membrane currents which could be associated with their firing behaviour. Finally, they offer a unique possibility for comparative investigation of different modes of firing adaptation (Fig. 1) which, according to previous studies on crayfish stretch receptors (Nakajima & Onodera 1969), must be regarded as encoder rather than as transducer properties.

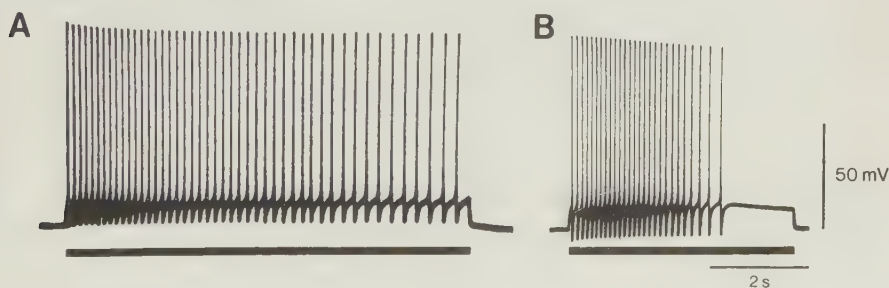


Fig. 1. Firing activity in a slowly (A), and rapidly adapting stretch receptor neurone (B) during constant electric stimulation (indicated by the horizontal bars). The slowly adapting receptor is able to maintain impulse activity throughout the stimulation, whereas the rapidly adapting receptor always terminates its impulse activity despite maintained stimulation.

Basically, the present study consists of the following parts: (a) a characterization of the cells' sub- and near-threshold membrane currents with respect to their ionic nature and kinetic properties; (b) the development of a receptor model on the basis of plausible kinetic principles and acquired empirical data; (c) a comparison of the functional behaviour of the living cells and the model inferred as a test of the validity of the latter; and (d) utilization of the model for a generalized description of the relation between encoder sensitivity and adaptation.

As main results of the investigation it was found that the receptor model inferred produces a fair simulation of the cells' functional activities and, therefore, may be considered as a valid theory of the encoder function. From an analysis of the model it appears that firing adaptation, in an early phase, is mainly due to a slow Na inactivation which can be more or less counteracted by a slow K inactivation and, in a later phase, mainly due to a Na^+ influx dependent pump current activation. It also appears that the difference in adaptation behaviour between slowly and rapidly adapting receptors is due to quantitative differences in some of the current controlling parameters whose influence on the spike generating mechanism decides whether the process of adaptation systematically will lead to a complete termination of the impulse activity, or only to a frequency reduction. Finally it will be shown that the encoder sensitivity may be entirely independent of firing adaptation.

METHODS

Preparations (I, II). All experiments were carried out on slowly and rapidly adapting nerve cells of stretch receptor organs from the second and third abdominal segments of the European lobster (*Homarus gammarus*). After isolation the stretch receptor organs were mounted in an experimental chamber which was continuously perfused with solutions of desired composition at 18°C. For achievement of optimal space clamp conditions, the afferent axon of the receptor neurone was in most preparations ligated at a distance of 600--800 μm from the cell body. After such treatment it was

concluded from theoretical considerations (Rall 1959) that a current control of at least 90% should be possible everywhere in the preparation. During experiments the receptor muscles were fully relaxed and measurements were begun only after the receptor neurones' membrane voltage and input resistance has stabilized completely following microelectrode impalement.

Solutions and drugs (I, II). As standard physiological solution was normally used a Tris buffered saline with the following composition (in mM): NaCl 325, KCl 5, CaCl_2 25, MgCl_2 4, MgSO_4 4, Tris HCl 26, Tris Base 4, and glucose 5. After bubbling with 5% CO_2 in O_2 the pH of the solution was adjusted to 7.2--7.4 by titrating with 0.5 mM NaOH. Solutions containing drugs as, for example, tetrodotoxin (TTX), tetraethylammonium chloride (TEA), and 4-aminopyridine (4-AP) were prepared fresh for each experiment.

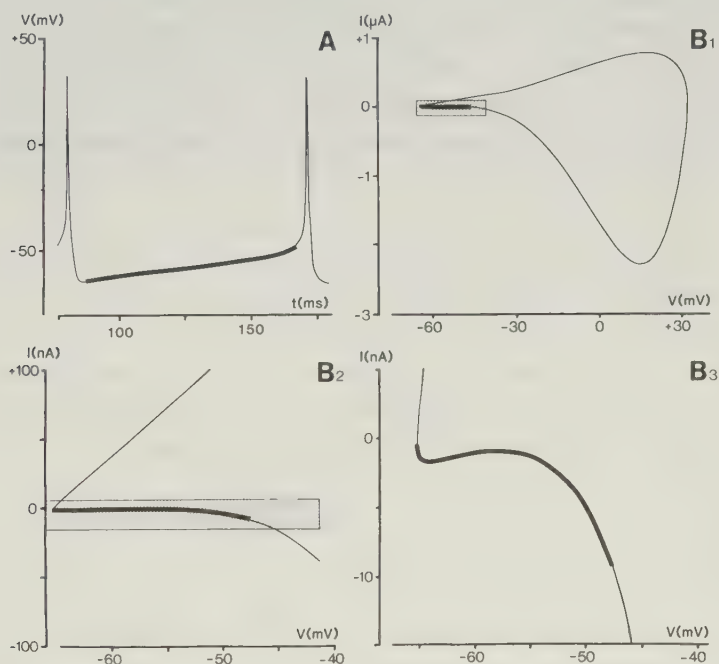


Fig. 2. A, action potentials of the slowly adapting receptor model. B₁--B₃, different magnifications of the current-voltage relationships during the same action potentials as in A. Note the great difference in current strength during the interspike discharge (thick line) and the action potential proper.

Voltage and current measurements (I, II). In most experiments membrane voltage and current measurements were done according to conventional electrophysiological techniques employing intrasomally placed KCl-filled double-barrelled microelectrodes with resistances of 6--8 M Ω . In preliminary tests it was found that in voltage clamp the recording device was adequate for measurement of membrane currents of up to ± 30 nA in a frequency range from DC to 500 Hz. The equipment was therefore considered suitable for registration of the weak and slow interspike discharge currents, but not of the spike currents proper (Fig. 2).

Measurements of intracellular ion concentrations (IV). Measurements of intracellular Na⁺ and K⁺ concentrations were performed using microelectrodes filled with either Na⁺ or K⁺ selective ion exchangers. Estimations of intracellular Cl⁻ were obtained from the Nernst equation following measurements of the Cl⁻ reversal voltage during spontaneous inhibitory transmitter release, or Cl channel activation by administration of γ -aminobutyric acid. The results of all measurements are given in terms of (free) concentrations, assuming that in extra- and intracellular compartments ionic strengths and, hence, activity coefficients are of comparable magnitudes.

Electronmicroscopy (V). For an electronmicroscopical investigation of the surface geometry of stretch receptor neurones, stretch receptor organs were isolated from the animals and mounted as for electrophysiological examinations. After this they were fixed in 2.5% glutaraldehyde and post-fixed in 1% OsO₄ in cacodylate buffer and, finally, prepared for electronmicroscopy according to conventional techniques.

Numerical methods (V). Mathematical modeling of the electrical activity of the receptors was made on a CAI Alpha LSI 4/90 computer, which has an accuracy of slightly more than 7 digits, using the single precision version of a program for numerical integration of partitioned stiff ordinary differential equations and differential-algebraic systems developed by Söderlind (1980).

RESULTS

The present investigation aims at the formulations of a comprehensive theory explaining the firing control exerted by the encoder mechanism in both slowly and rapidly adapting stretch receptor neurones. For this purpose a number of assumptions or hypotheses were drawn up and experiments were designed and performed in order to test the hypotheses, and to find the parameter values for substitution into the mathematical expressions of the theory inferred. As main result of this study it was found that the same basic concepts could be used for the description of firing control in slowly and rapidly adapting cells, and that differences in firing behaviour could be referred to quantitative differences between some of the parameters involved. This fact is summarized in the APPENDIX, where the parameter values for the two cell types are listed together with the mathematical expressions chosen as components in a model of neuronal encoder function.

Below the findings made in parallel measurements on slowly and rapidly adapting cells are given in greater detail.

Identification of membrane currents (I)

Different membrane currents were identified by their drug sensitivity and operationally defined as loss in total membrane current due to drug application. In one case (the C-current) the current was identified by its ion dependence, but defined by its dynamic properties. A remaining drug insensitive current was regarded as the leak current.

According to the definitions given the same type of currents were found in both slowly and rapidly adapting cells. These currents were (a) a TTX sensitive Na^+ current, (b) a TEA + 4-AP sensitive K^+ current, (c) a ouabain sensitive pump current, (d) a Ca^{++} dependent K^+ current (C-current), and (e) a drug insensitive leak current.

For the TEA + 4-AP sensitive K^+ current it holds that it was defined as one entity, since the current blocked by the combination of drugs was larger

than the current portions blocked by either of the drugs alone and, therefore, neither of these current portions could be regarded as pharmacologically independent.

The existence of a pump current was evidenced by an instantaneous loss of outward current following application of ouabain and, also, by the activation of an outward current during pump stimulation following intracellular Na^+ accumulation (see below).

For the leak current it was concluded that it is carried by Na^+ , K^+ , and Cl^- in combination, since variation in the external concentrations of these ions led to changes in the leak current reversal voltage. The leak current was also regarded as a time independent entity whence, after blockage of the TTX sensitive Na^+ current and TEA + 4-AP sensitive K^+ current, its responses to sudden changes in membrane voltage were considered equivalent to the resulting jumps of the total membrane current.

The Ca^{++} dependent K^+ current (C-current) was seen to occur in conditions of prolonged suprathreshold depolarization after treatment with TTX, TEA, and 4-AP. The activation of this current, and its decay following repolarization, were slow processes following an exponential time course with a time constant of 200--300 ms. Since this current was not identifiable in the absence of 4-AP and no phenomena of firing regulation could be associated with its kinetic properties it will no longer be considered in the further discussion. Tentatively, the C-current existence can be explained as a pharmacological artifact due to the presence of 4-AP, which in molluscan neurones has been shown to stimulate the generation of a Ca^{++} dependent K^+ current (Hermann & Gorman 1981).

A more detailed account of the kinetic properties of the various currents identified will be given below. However, an example of the stationary voltage dependence of the membrane currents of a slowly and rapidly adapting cell is shown in Fig. 3. For isolation of the currents, TTX was applied in 480 nM, TEA in 10 mM, 4-AP in 0.5 mM, and ouabain in 5 mM concentrations. The figure illustrates the fact that, mainly as result of differently adjusted ratios between the TTX sensitive Na^+ current and TEA + 4-AP sensitive K^+ current, the relationship between membrane voltage and total ionic current has, in depolarizing voltage regions, usually a negative slope in slowly, but never in rapidly adapting cells. This

observation agrees with the finding that, in order to achieve impulse initiation, a minimum pace of depolarization is needed in the rapidly, but not in the slowly adapting receptor.

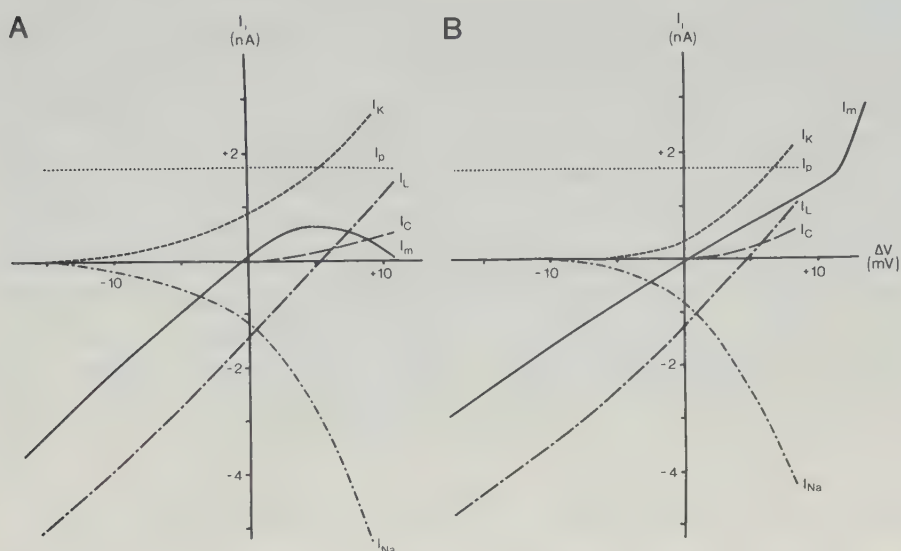


Fig. 3. Relationships between membrane voltage, (ΔV), and individual membrane currents, I_i , in a slowly (A), and rapidly adapting cell (B). The membrane voltage is given relative to its resting values at -68 mV in A, and -67 mV in B. I_m , total membrane current; I_{Na} , TTX sensitive Na^+ current; I_K , TEA + 4-AP sensitive K^+ current; I_C , Ca^{++} dependent K^+ current; I_p , ouabain sensitive pump current; and I_L , drug insensitive leak current.

Membrane current kinetics (II, III, VI)

It is from the above clear that the same membrane currents exist in both slowly and rapidly adapting receptor neurones. From this it follows that differences in the cells' firing responses have to be attributed to quantitative differences in the individual current kinetics, rather than to qualitative differences in the cells' current equipment.

The various passive currents are thought to pass through the membrane in accordance with the constant field assumption (Goldman 1943). Hence their strengths and signs will be explicitly dependent on the membrane voltage, extra- and intracellular ion concentrations, and the membrane's ion permeabilities [Eqs. (A2)--(A6)]. For the TTX sensitive Na^+ current and TEA + 4-AP sensitive K^+ current it is also assumed that they pass through membrane channels whose permeability is controlled by dynamically voltage

dependent gating variables. Mathematically, the gating function is expressed by so-called gating variables (Hodgkin & Huxley 1952). In the present formulations these gating variables are all considered to be independent of each other, although there is increasing evidence in the literature that the rapid activation (m) and inactivation (h) of the Na channel are coupled to each other (for references, see Armstrong 1981). However, so far this possibility has not been mathematically expressed in a form suitable for incorporation into a comprehensive membrane model. It is also likely that, when such an expression is available, its practical effect in a complete membrane model will be much less than its theoretical impact, since with the classical formulations implying uncoupled gating mechanisms it has been possible to achieve excellent simulations not only of the action potential itself, but also of more complex phenomena like accommodation (Frankenhaeuser & Vallbo 1965). In the present membrane model the dynamic voltage dependence of all gating variables have been put in a mathematical form [Eqs. (A9)--(A12)] which represents a slight reformulation (see APPENDIX in paper II) of the state transition theory proposed by Tsien & Noble (1969) and later used by Keynes & Rojas (1976).

Na⁺ current kinetics. In both receptors the Na⁺ current dynamics were characterized by fast as well as slow reactions. The fast reactions took place in connexion with depolarizing and repolarizing voltage steps and consisted of a fast turning on, and turning off, respectively, while the slow reaction corresponded to a slow inactivation appearing during maintained depolarization.

The fast reactions were assumed to be controlled by the "classical" (Hodgkin - Huxley) types of fast Na activation (m) and inactivation (h). Both of these processes are too fast for dynamical resolution with the clamping technique available. However, current measurements reflecting their combined stationary voltage dependence in sub- and near-threshold voltage regions could be obtained (Fig. 4A). This information was considered valuable, since it could be used as an aid for the proper positioning of the stationary m_{∞} - and h_{∞} -voltage relationships along the voltage scale. The exact form of these relationships as well as the relaxation times of the m - and h -processes underlying the generation of action potentials had to be inferred from relevant data in the literature (Hodgkin & Huxley 1952, Frankenhaeuser & Huxley 1964) in combination with a

trial-and-error procedure including the achievement of successful mathematical reproduction of the individual action potential.

The slow Na inactivation, I_1 , resembled in its kinetic behaviour similar reactions previously described in both vertebrate (Fox 1976, Brismar 1977) and invertebrate (Adelman & Palti 1969, Schaaf et al. 1976, Rudy 1978, 1981) peripheral nerve. It was therefore considered as a genuine gating reaction which, also, could be shown to be Ca^{++} dependent, like the classical fast gating reactions of the Na channel in both invertebrate (Frankenhaeuser & Hodgkin 1957, Julian et al. 1962) and vertebrate (Hille 1968) nerve preparations. Having the properties of a first-order reaction the slow Na inactivation could, in analogy with the fast Na gating reactions, be characterized by the voltage dependence of its stationary value, I_{∞} , and its relaxation time, τ_1 (Fig. 4B and C), and was included in the formal description of the gated Na^+ current [Eq. (A2)].

From a comparison of the measurements made on slowly and rapidly adapting cells (Fig. 4) it seems that the slow Na inactivation, I_1 , is similarly adjusted in the two cell types, whereas the Na^+ current, I_{Na} , is, in subthreshold regions, less activated in the rapidly than in the slowly

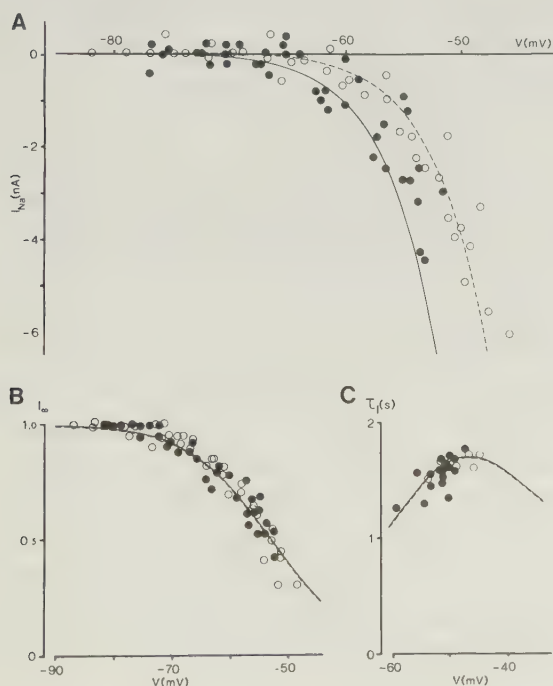


Fig. 4. Kinetic properties of the Na^+ current in slowly (●) and rapidly adapting cells (○). A, relationships between membrane voltage, V , and Na^+ current, I_{Na} , in the absence of slow inactivation. B, relationships between membrane voltage, V , and stationary value of the slow Na inactivation, I_{∞} . C, relationships between membrane voltage, V , and relaxation time, τ_1 , of the slow Na inactivation. All curves are computed in accordance with the mathematical formulations of the slowly (—) and rapidly adapting stretch receptor models (-----), as given in the APPENDIX.

adapting receptor. In the models (smooth curves) this is accounted for by assuming that the m-system is placed in more positive voltage regions in rapidly than in slowly adapting cells. In addition a larger value had to be assumed for the maximum Na^+ permeability in the former than in the latter cell, in order to obtain good agreement between simulated and recorded action potentials (cf. Fig. 9).

K⁺ current kinetics. In both receptor types the dynamics of the K^+ current resembled the dynamics of the Na^+ current by displaying a fast activation followed by a slow inactivation when the cells were exposed to a depolarizing voltage step. The fast activation was assumed to be controlled by the receptors' counterpart of the Hodgkin-Huxley n-system. By measuring the K^+ current proportional to the stationary value of n in sub- and near-threshold voltage regions (Fig. 5A) it was possible to find a proper positioning of the n_{∞} -voltage relationship along the voltage scale. The exact form of this relationship and the relaxation time of the n-process were inferred from data in the literature and by a trial-and-error method involving mathematical simulation of the action potential.

The slow K inactivation, r, seemed to follow an exponential time course and was therefore regarded as a first-order process. With respect to its nature, the possibility had to be considered that a decrease in K^+ current resembling K inactivation might result from pericellular K^+ accumulation, as has previously been observed in various kinds of nerve and muscle preparations (Frankenhaeuser & Hodgkin 1956, Neher & Lux 1973, Baumgarten & Isenberg 1977, Nicholsson et al. 1978, Cleemann & Morad 1979, Brown et al. 1980, Moran et al. 1980). In this case the redistribution of K^+ should have manifested itself by dynamic changes of the leak current, which is carried by K^+ to a considerable extent, and by a distinct tail of inward current on termination of the membrane depolarization. Since neither of these effects were ever observed in the experiments and, furthermore, direct measurements with K^+ sensitive electrodes showed only minor accumulation of pericellular K^+ during intense firing activity, the slow K inactivation was regarded as a genuine gating process. This view is also supported by similar conclusions in the literature based on observations of slow processes of K inactivation in both vertebrate and invertebrate nerve preparations (Frankenhaeuser & Waltman 1959, Ehrenstein & Gilbert 1966, Nakajima &

Onodera 1969, Connor & Stevens 1971a, Schwartz & Vogel 1971). In agreement with this view the slow K inactivation was, like the slow Na inactivation, characterized by the voltage dependence of its stationary value, r_{∞} , and relaxation time, τ_r (Fig. 5B and C), and included in the formal description of the K^+ current [Eq. (A4)].

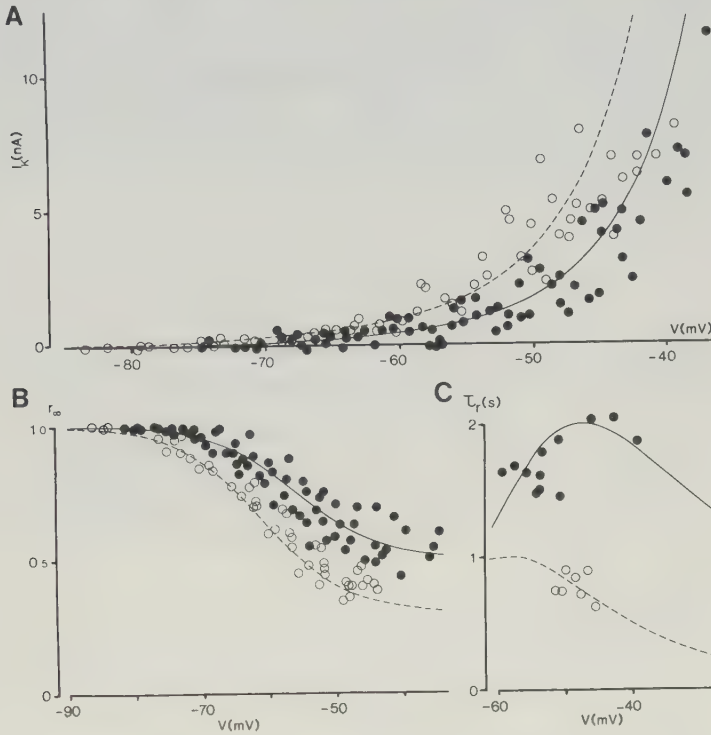


Fig. 5. Kinetic properties of the K^+ current in slowly (●) and rapidly adapting cells (○). A, relationships between membrane voltage, V , and K^+ current, I_K , in the absence of slow inactivation. B, relationships between membrane voltage, V , and stationary value of the slow K inactivation, r_{∞} . C, relationships between membrane voltage, V , and relaxation time, τ_r , of the slow K inactivation. All curves are computed in accordance with the mathematical formulations of the slowly (—) and rapidly adapting receptor models (-----), as given in the APPENDIX.

By comparing the measurements made on slowly and rapidly adapting cells (Fig. 5) it was possible to infer that the activation of K^+ current, I_K , with depolarization assumed proportional values in the two cell types. In this case the differences in amount of current activated can be ascribed to a larger maximum K^+ permeability in the rapidly than in the slowly adapting cell, while the n -system can be assumed to be similarly adjusted in both receptors. A more pronounced difference seems to exist with respect to the

r-process which is both faster and, also, becomes more complete at strong depolarizations in the rapidly than in the slowly adapting cell.

Leak current kinetics. As the leak current could be shown to consist of a Na^+ , K^+ , and Cl^- component, each of these components was treated as a separate current obeying constant field kinetics [Eqs. (A3), (A5), and (A6)]. The individual leak permeabilities were inferred from the slope conductance, $(g_L)_{\Delta V_L}$, of the leak channel around resting voltage. In the experiments it was found that this slope conductance is systematically larger for rapidly than for slowly adapting receptors.

Intracellular ion control (IV)

Stationary conditions. In resting conditions intracellular ion concentrations were measured in slowly as well as in rapidly adapting receptor neurones. It appears from these measurements (Table 1) that the ion concentrations are identical in the two cell types, although there is a fairly wide scatter in the estimations. Partly, this scatter may be due to a variation in degree to which the cells were disturbed by the microelectrode impalement.

Table 1. Resting values of intracellular ion concentrations. Mean $\pm$ S.D.

$[\text{Na}^+]_i$ and $[\text{K}^+]_i$ were determined with ion sensitive microelectrodes and $[\text{Cl}^-]_i$ from measurements of the Cl^- reversal voltage using KCl-filled electrodes.

	$[\text{Na}^+]_i$ (mM)	$[\text{K}^+]_i$ (mM)	$[\text{Cl}^-]_i$ (mM)
Slowly adapting cell	20.4 ± 6.3 (n = 10)	156.0 ± 41.9 (n = 8)	$50.5 \pm 8.5^*$ (n = 11)
Rapidly adapting cell	20.4 ± 3.2 (n = 6)	155.0 ± 13.0 (n = 3)	$46.3 \pm 12.4^{**}$ (n = 6)

* including measurements of post-synaptic miniature current reversal voltage in 4 cells.

** including measurements of post-synaptic miniature current reversal voltage in 5 cells.

Excited conditions. In excited conditions ion measurements were performed in slowly adapting cells only. For Na^+ and K^+ determinations, membrane excitation was accomplished by stretch stimulation giving rise to impulse firing which, after adaptation, was maintained at stationary frequencies of 7--10 Hz. As result of such stimulations it was found that there was a slow

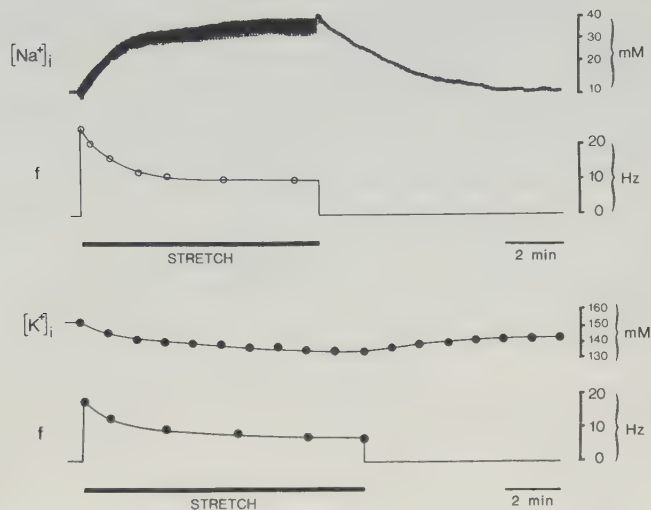


Fig. 6. Measurements of intracellular Na^+ concentration, $[\text{Na}^+]_i$, and K^+ concentration, $[\text{K}^+]_i$, during repetitive firing at frequencies, f , in two different slowly adapting cells. The firing activities were evoked by stretch stimulation, as indicated by the horizontal bars.

decrease of intracellular K^+ and a slow increase in intracellular Na^+ which, on cessation of the stimulation, were completely reversible (Fig. 6). Since in stationary conditions the concentration changes led to new stable intracellular ion values, it follows that the passive fluxes of Na^+ and K^+ are matched by equally large active transports which are assumed to be driven by the cell's Na-K pump.

With respect to the intracellular Cl^- concentration it was observed that during prolonged stimulation by current injection through KCl-filled microelectrodes it remained rather constant. Since KCl-filled electrodes were routinely used throughout the investigation and, furthermore, very little is known about the mechanisms of intracellular Cl^- control, the intracellular Cl^- concentration will be regarded as a constant quantity.

Pump current kinetics. By frequently shifting between depolarizing current clamp and voltage clamp at resting voltage it could be demonstrated that during prolonged periods of repetitive firing there is a slow activation of outward current which shows no signs of voltage dependence (Fig. 7). The activation of this current is effectively suppressed by application of ouabain, substitution of Li^+ for Na^+ in the external solution, or prevention of transmembrane Na^+ influx by exposure to TTX. It is therefore regarded as an increase in pump current production due to stimulation of an electrogenic Na-K pump by intracellular Na^+ accumulation.

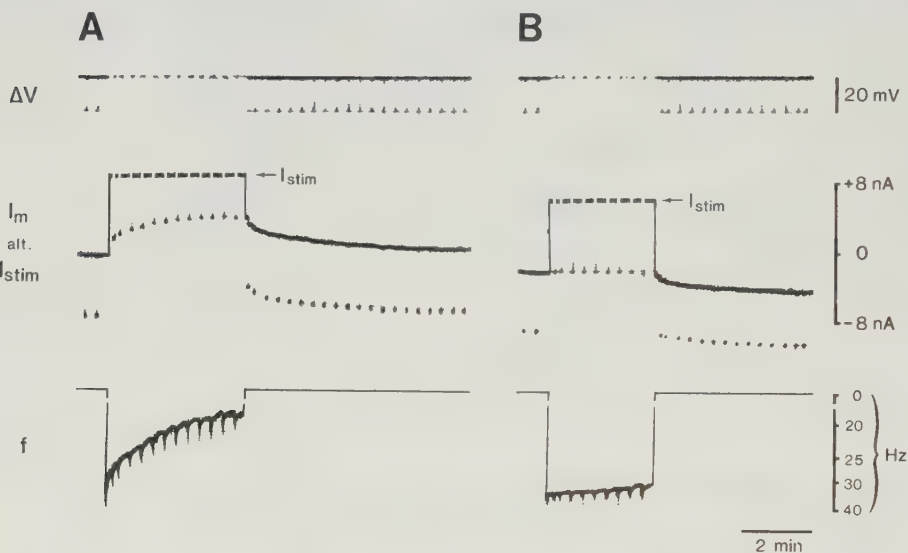


Fig. 7. Recordings of membrane voltage, ΔV (relative to resting voltage at -60 mV), membrane current, I_m , in alternation with intracellularly injected stimulating current, I_{stim} , and firing frequency, f , in a slowly adapting cell before (A) and 6 min after (B) treatment with 0.5 mM ouabain. The current measurements were performed at resting voltage (of the pharmacologically untreated cell) and during short pulses of membrane hyperpolarization after the impulse activity. Impulse firing was evoked by depolarizing current injection which was interrupted for short (0.5–1 s long) periods of membrane current measurements. Before ouabain treatment the recordings show that during impulse firing there is a slow activation of outward current which slowly declines during a period of post-tetanic recovery. The current activated is ouabain sensitive and voltage independent as appears from the constant height of the current transients evoked by the post-tetanic pulses of membrane hyperpolarization; it is therefore regarded as a pump current increment. In the voltage recordings blank segments correspond to impulse firing during which pen deflections were too rapid to be properly registered. For greater graphical clearness the gaps in the recordings of the stimulating current (coinciding with the recordings of membrane current) have been widened and the pen traces to and from zero frequency in connexion with the short membrane current measurements have been removed.

Following this conclusion the pump current was described as a function of intracellular Na^+ in accordance with saturation kinetic principles (Hill 1910) assuming a Hill coefficient of 3 (Nelson & Blaustein 1980) [Eqs. (A7) and (A8)]. In this description the external K^+ concentration is not given as an explicit variable since, in previous experiments (see above) it has been found to undergo very little kinetic variations. The internal concentrations of Na^+ and K^+ were obtained by integration of the continuity equations for the ions involved [Eqs. (A13) and (A14)].

For a determination of the Na-K pumping ratio, stationary values of pump and Na^+ current activations, as inferred from depolarizing voltage clamp measurements before and after treatment with TTX, were divided by each other (Fig. 8A). As a result a quotient was found which closely corresponded to a 3:2 Na-K pumping ratio. Since this ratio is also commonly referred to in the literature (cf. Thomas 1972) it was adopted in the present pump current model [Eq. (A8)].

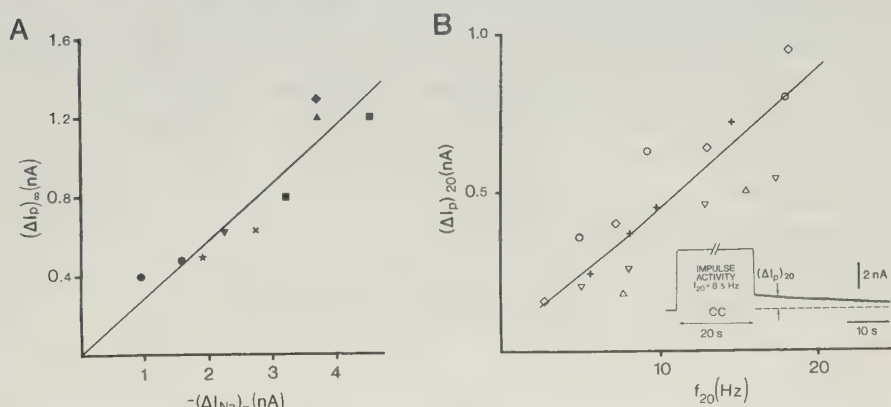


Fig. 8. A, stationary relationship between depolarization dependent Na^+ current, (ΔI_{Na}) , and corresponding pump current, (ΔI_p) , increments in 7 different slowly adapting cells (different symbols). The solid line was obtained by least square estimation of the slope. B, inset, recording of membrane current in voltage clamp before and after a 20 s long pulse of depolarizing current clamp, CC, giving rise to repetitive impulse activity in a slowly adapting cell. The interrupted line indicates the level of zero membrane current at resting voltage level (-67 mV). f_{20} is the firing frequency at the end of the impulse train and $(\Delta I_p)_{20}$ the corresponding pump current increment. Graph, relationship between firing frequency at the end of 20 s long pulses of depolarizing current clamp, f_{20} , and corresponding pump current increments, $(\Delta I_p)_{20}$, in 6 different slowly adapting cells (different symbols). The solid line corresponds to a theoretical relationship computed from the slowly adapting receptor model, as given in the APPENDIX.

During current clamp experiments at maximum possible stationary firing frequencies (about 30 Hz) pump current increments of 6--7 nA could be observed. For the pump to retain full regulatory power (gain) even under such circumstances and, in particular, in conditions of stretch stimulation during which the Na^+ influx is further increased by activation of the cell's own generator mechanism, its maximum current production capacity should be significantly larger than the values observed. A maximum pump current of 20 nA was therefore assumed for the slowly adapting cell. In Fig. 8B it is shown that with this assumption the mathematical model of the slowly adapting receptor is able to give a fair reproduction of empirical data concerning the relation between firing frequency at the end of 20 s long spike trains and the corresponding pump current activation.

Theoretically, it was expected that, because of its smaller size (see below), the rapidly adapting stretch receptor neurone should accumulate more intracellular Na^+ for a given amount of impulse activity than the slowly adapting cell and, therefore, also exhibits a higher degree of Na^+ influx dependent pump current activation. Experimentally, it proved however that the development of outward current increments, indicative of pump current activation, was much weaker than expected. Although partly this finding could be ascribed to a certain loss of intracellular K^+ it still seemed justified to conclude that in the rapidly adapting receptor there was a genuinely low sensitivity of the pump mechanism to changes in intracellular Na^+ concentration. In the model this possibility is expressed by assuming a comparatively low maximum pump current value.

The mathematical receptor model (V, VII)

The membrane equation. In the living receptor neurone action potentials are set up in the axonal spike-trigger zone, from where they invade the cell's soma-dendrite region (Grampp 1966). Hence, any simulation of the cell's firing control will of necessity represent a simulation of the functional behaviour of the cell's spike-trigger zone. Since, basically, the process of spike initiation in the spike-trigger zone is independent of impulse conduction, the Hodgkin-Huxley (1952) membrane equation for the non-propagated electrical activity was regarded as an adequate basis for the receptor model in this investigation [Eq. (A1)].

Complementary assumptions. From anatomical data of Alexandrowicz (1951a, b) and Florey & Florey (1955) as well as from own (unpublished) observation it was inferred that slowly and rapidly adapting receptor neurones have cell volumes of $250.000 \mu\text{m}^3$, and $100.000 \mu\text{m}^3$, respectively, and that both cell types have the same projected surface area of $60.000 \mu\text{m}^2$. By using this value in combination with measurements on exponential voltage transients evoked by intracellular applied current steps the membrane capacitance was in both receptor neurones found to be $13 \mu\text{F}/\text{cm}^2$. When judging this estimate it has to be kept in mind that because of a pronounced, electronmicroscopically observable membrane folding the true size of the surface membrane is about three times larger than its projected value.

In both receptor types the resting concentrations of intracellular Na^+ and K^+ were set to 20 mM, and 160 mM, respectively, while the intracellular Cl^- concentration was maintained at an invariable value of 46 mM. This value corresponds to a Cl^- reversal voltage of -55 mV which is artificially high as result of Cl^- leakage from the KCl-filled microelectrode, but which was nevertheless chosen, since it conforms with the experimental facts with which the models are compared. The extracellular ion concentrations were assumed to equal those of the perfusing solution.

Although the receptors showed a wide variety in resting voltages their median values were both approximately -65 mV. This voltage was therefore chosen when adjusting the models to steady state conditions by solving the equation system (A15)--(A18) in the APPENDIX.

Simulation of firing activity (V, VII)

Following adjustment to resting conditions dynamic responses of the receptor model were obtained by solving Eq. (A1) after application of a signal simulating constant current stimulation.

Individual action potentials. Fig. 9 shows examples of recorded (upper panels) and simulated (lower panels) action potentials and their time derivatives obtained initially during low frequency spike trains produced by both slowly (A) and rapidly (B) adapting cells and their respective models. It is seen that the simulations reproduce the recordings faithfully

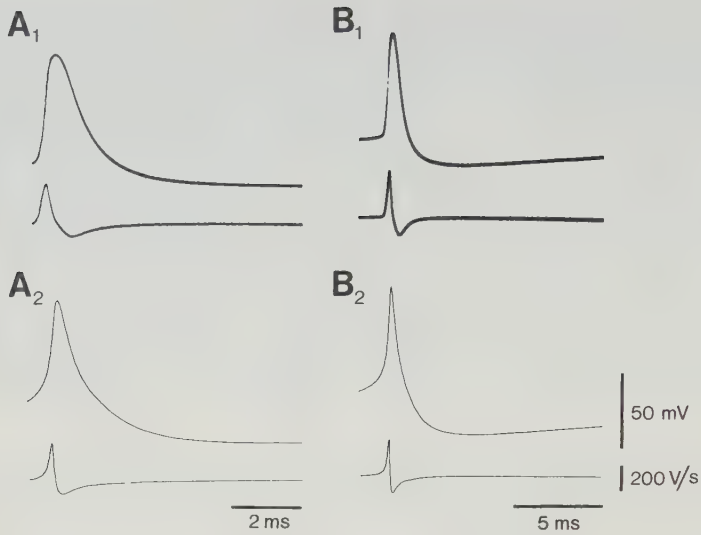


Fig. 9. Upper panels, intrasomally recorded action potentials (upper traces), and time derivatives (lower traces) of a slowly (A₁), and rapidly adapting cell (B₁). Lower panels, mathematical simulations of action potentials and their time derivatives for the slowly (A₂), and rapidly adapting stretch receptor model (B₂). Both recordings and simulations were obtained in the beginning of low frequency spike trains.

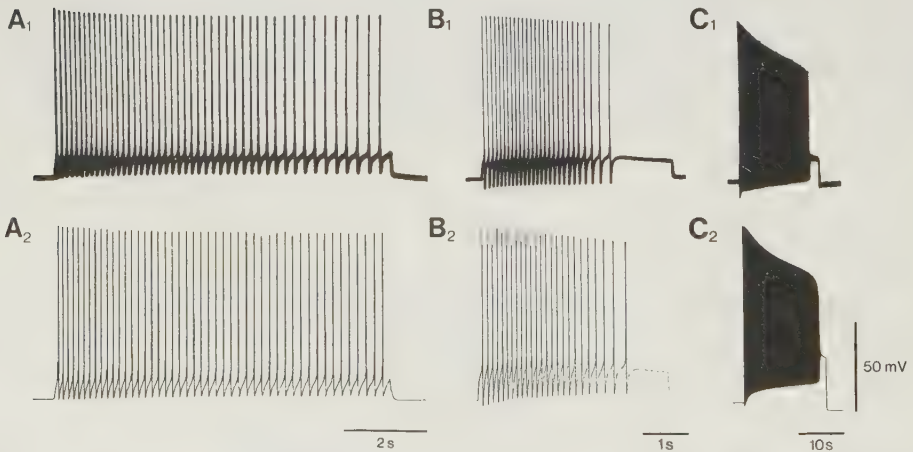


Fig. 10. Upper panels, intrasomally recorded spike trains of a slowly adapting receptor (A₁), and of a rapidly adapting cell in conditions of weak (B₁), and strong (C₁) current stimulation. Lower panels, mathematical simulation of firing activity in similar circumstances as above for the slowly (A₂), and rapidly adapting receptor models (B₂, C₂).

with respect to impulse height, duration, and derivative amplitude. The simulated action potentials differ, however, from the recorded ones by showing a more gradual onset of their upstrokes. This can be related to the fact that the models reproduce an ideal pace-maker activity, while the intrasomal recordings represent a propagated impulse activity.

Repetitive firing. An illustration of recorded and simulated spike trains of slowly (A) and rapidly (B) adapting receptor neurones and their respective models is given in Fig. 10. It is shown that the simulations (lower traces) are fair reproductions of the recordings (upper traces) displaying both a typical decline in spike height, decrease in depth of the spike after-hyperpolarization, and the abrupt termination of the impulse activity in the rapidly adapting cell. In the models the mechanisms responsible for the gradual changes in spike configuration could be identified by setting the slow l and r systems to constant values one at a time. As a result it was found that the reduction in spike height is secondary to the slow Na inactivation (l), while the decrease in after-hyperpolarization is caused primarily by the slow inactivation of the K channel (r).

Firing adaptation. For a better comparison of the adaptation behaviour of the living cells and their models, frequency-time relationships were obtained for different stimulation strengths both experimentally and mathematically. The relationships are compiled in Fig. 11, where the empirical data, recorded in individual cells, are represented by solid, and the theoretical data by interrupted lines. Again, it is seen that the models give a satisfactory reproduction of the course of adaptation.

In the slowly adapting cell (panel A in Fig. 11) the adaptation is relatively rapid initially but decreases successively during maintained stimulation, and it is observed that neither the living cell nor the model has reached a steady state after 20 s of stimulation. By keeping the different adaptive mechanisms still one at a time it was revealed that the initial fast adaptation is caused by the slow Na inactivation, while the later slow phase is mainly due to Na^+ dependent pump current activation.

In contrast to the slowly adapting neurone many rapidly adapting cells display an initial transient increase in firing frequency before the adaptive mechanisms take over and gradually reduce the frequency, until

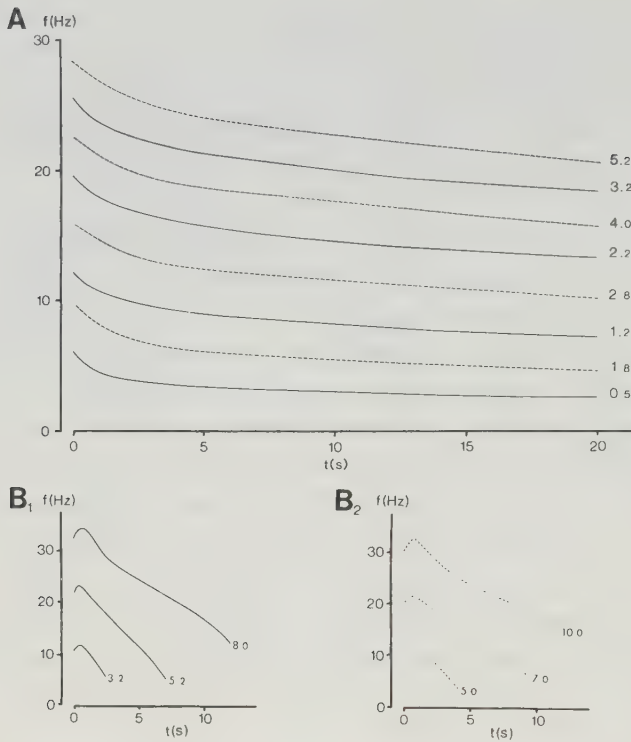


Fig. 11. A, firing frequency, f , as a function of time, t , in a slowly adapting cell (solid lines), and in the corresponding mathematical model (interrupted lines) after sudden application of constant prolonged stimulating currents. Lower panels, same relationships as in A for a rapidly adapting cell (B₁), and the rapidly adapting receptor model (B₂), where the end points of the curves coincide with the abrupt termination of impulse firing. Stimulation strengths are given in nA beside each curve.

impulse firing is suddenly stopped (panels B). The cause of this behaviour was investigated in the same way as before by keeping the adaptive mechanisms constant one at a time. As a result it was found that the initial frequency increase is an effect of the slow K inactivation which, temporarily, may dominate over the slow Na inactivation. The effect of the latter process is to successively lower the amount of activatable Na⁺ current, thereby initiating a regenerative cycle of spike accommodation. In connexion herewith the firing frequency is gradually reduced to levels below which a repetitive activity is no longer possible. As shown in Fig. 12 these levels are largely determined by the adaptation parameter, l , for a given adjustment of the spike generating n , m , and h systems as well as of the maximum Na⁺ and K⁺ permeabilities. From the analysis of Fig. 12 it follows that for the rapidly adapting cell, with its small ratio between

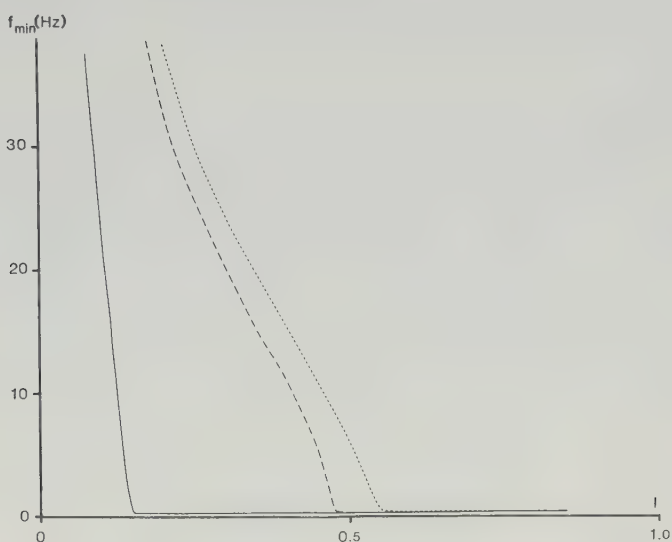


Fig. 12. Minimum firing frequency, $f_{\min}$, as a function of the slow Na inactivation, l , for different receptor models. Slowly adapting model (—); rapidly adapting model (-----); rapidly adapting model where the h_{∞} voltage relationship has been moved 2 mV in a depolarizing direction (---).

near-threshold Na^+ and K^+ currents, relatively minor decreases in l during firing adaptation are sufficient for the firing frequency to reach its cut-off level. It also follows that this level increases with increasing stimulation intensity and resulting firing frequency (Fig. 11), since with higher initial firing frequencies adaptation can continue for longer periods of time during which l may drop to lower values than during short spike trains at low stimulation intensities. Finally, it appears from Fig. 12 that with minor variations in adjustment of the spike controlling parameters (such as the midpoint value of the h_{∞} relationship in the rapidly adapting model) there may be major changes in minimum possible firing frequency. This relation explains why the ability to maintain prolonged repetitive firing is found to vary considerably among rapidly adapting cells.

In summary, it may be stated that in the rapidly adapting receptor adaptation is mainly caused by the slow Na inactivation leading to an abrupt firing termination, while the Na^+ influx dependent pump current activation is only of secondary importance, since the spike trains are

usually too short to give rise to an appreciable intracellular Na^+ accumulation.

Stimulus sensitivity. For another type of comparison between firing characteristics displayed by living cells and their respective models, frequency was plotted as a function of stimulating current. For slowly adapting cells such plots could be obtained at different instances from application of the stimulation (Fig. 13A), whereas for rapidly adapting cells this was meaningful only for the initial spikes because of a wide scatter in adaptation behaviour (Fig. 13B). A comparison between empirical data (solid lines) and theoretical relationships (interrupted lines) shows that the models faithfully reproduce the cells' stimulus sensitivity, defined as the slope of the curves at comparable firing frequencies. The fact that the two sets of curves are horizontally displaced from each other signifies differences in rheobase values between the living cells and the mathematical models. These differences can be attributed to chance dissimilarities with respect to resting voltage and/or leak permeability in the entities compared. Similar differences in parameter adjustment probably also explain why it is often seen that the rheobase may vary considerably between cells which are remarkably alike each other as far as their stimulus sensitivity is concerned.

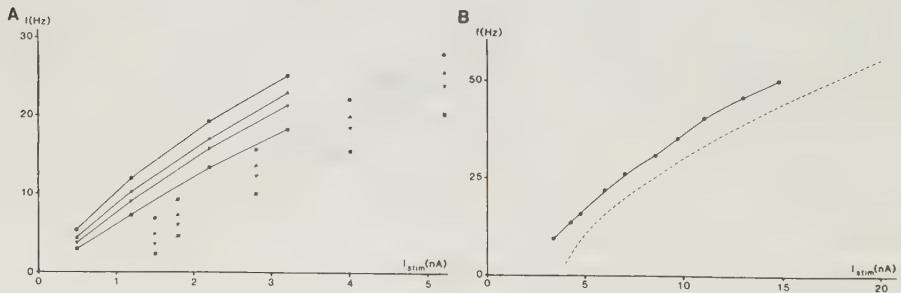


Fig. 13. A, firing frequency, f , as a function of stimulating current, I_{stim} , in the same slowly adapting cell as in Fig. 11 (solid lines), and in the slowly adapting mathematical model (interrupted lines). The measurements were obtained 0.2 s (●), 2 s (▲), 5 s (▼), and 20 s (■) after sudden application of the stimulating current. B, same relationships as in A for the first two impulses of a rapidly adapting cell (solid line), and the corresponding mathematical model (interrupted line).

DISCUSSION

In the RESULTS it has been shown that the proposed receptor model adequately describes the firing behaviour of the slowly and rapidly adapting receptors with respect to their stimulus dependence (sensitivity), time dependence (adaptation), and general shape of the action potential. From an analysis of the model it could also be concluded that the different firing properties displayed by the two receptor types are attributable to quantitative differences in some of the current control parameters. Among other things these differences imply that the ratio between near-threshold Na^+ and K^+ currents is markedly higher in slowly than in rapidly adapting cells and that, therefore, the initiation of action potentials is more easily disturbed by accommodative processes in the latter than in the former cells. From the model it is also clear that firing adaptation is primarily due to slow Na inactivation and a slow Na^+ influx dependent pump current activation. However, the latter process plays a significant role only in slowly adapting cells which, because of their insensitivity to accommodation, are able to fire long enough for the intracellular Na^+ accumulation to become appreciable.

With regard to the close correspondence between cell and model behaviour it seemed appropriate to use the model as a basis for further analysis of the principles of firing control exerted by the encoder mechanism. In particular, it was considered relevant to investigate the relation between stimulus sensitivity and adaptation which, at a superficial glance, may appear intimately linked to each other (cf. Fig. 13A).

Stimulus sensitivity

Stimulus sensitivity has above been described as relation between stimulus intensity and resulting firing frequency. However, more exactly it should be defined as the instantaneous change in firing frequency per step change in stimulation intensity. In this way sensitivity becomes a time independent property which can be seen as quite distinct from adaptation and, also represent a more adequate characterization of the cell's dynamic responsiveness. In the present analysis time independence of the sensitivity

was achieved by keeping the parameters of the adapting systems (slow Na and K inactivations, and intracellular ion concentrations) constant at desired levels following adjustment of the models to resting conditions (see RESULTS). In the living cell this procedure closely corresponds to measuring only frequencies of the first two spikes after a given step change in stimulation intensity.

Firing threshold. By inspection of a repetitive spike train (cf. Figs. 1 and 2) it can be seen that the repetitive cycle consists of a slow phase of interspike discharge followed by the action potential proper. Since the duration of the action potential is short and subject to relatively small variations, it is mainly the time course of the slow phase of interspike discharge that determines the frequency of an impulse train. Hence, it should be possible to deduce the firing frequency by finding a relationship between the stimulating current and the duration of the slow phase. In the literature (cf. Skaugen 1975) such analyses are usually based on the assumption that the beginning and end of the slow phase are located at two fixed voltage levels. However, by comparing an adapted with an unadapted simulated spike train (Fig. 14) it is seen that this definition does not always hold since interspike discharges of similar rates can be placed in entirely different voltage regions. This observation indicates that the notion of the firing threshold as a fixed voltage level at which the Na^+ current starts to dominate and the action potential commences is not always

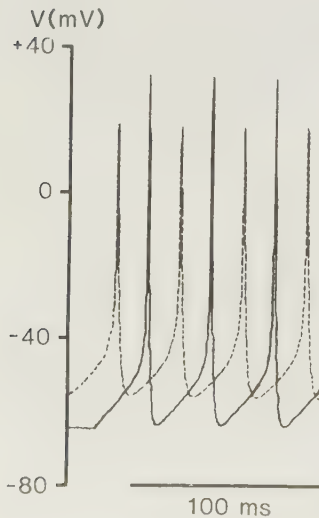


Fig. 14. Repetitive spike trains of the slowly adapting receptor model obtained initially during stimulation of 5.8 nA (solid line), and after 20 s of adaptation during stimulation of 8 nA (interrupted line). Note that the firing frequencies of the two spike trains are identical although the interspike discharges take place in entirely different voltage regions.

meaningful. A more general and, therefore, more useful concept of the slow phase is obtained by defining it as the interval between the bottom of the afterhyperpolarization and a certain rate of voltage increase at the beginning of the action potential. The advantage with this definition is that the boundary conditions now relate to certain time derivatives of the membrane voltage and not the membrane level itself. Since furthermore, in accordance with the membrane equation [Eq. (A1)] every voltage derivative corresponds to a certain membrane current, the above concept can also easily be put in a mathematical form (see below).

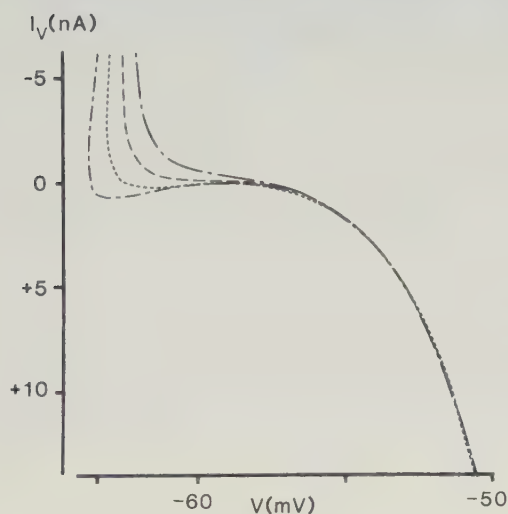


Fig. 15. Relationships between membrane voltage, V , and the voltage dependent membrane current, I_V , during interspike discharges (cf. Fig. 2) in the slowly adapting receptor model at stimulation strengths of 2 nA (---), 4 nA (-·-·-), 6 nA (---), and 8 nA (—).

Interspike discharge kinetics. From previous analysis the relationship between membrane voltage and membrane current (Fig. 2) it can be seen that the slope conductance during the slow phase initially is approximately zero whereas it turns increasingly more negative as the firing threshold is approached. With respect to the membrane current this circumstance corresponds to an unstable situation involving a positive feedback loop which in its simplest form can be described in the following way:

$$\frac{dI_V}{dt} = aI_V + bI_d, \quad (1a)$$

where

$$I_d = I_{stim} - I_r. \quad (1b)$$

In these expressions a and b are constant coefficients, while I_V denotes a voltage dependent membrane current arising when the intracellularly injected stimulating current, I_{stim} , exceeds a rheobase value, I_r . Note that for convenience of graphical representations depolarizing currents will in the following be given as positive quantities.

From mathematical reproductions of I_V in interspike voltage regions (Fig. 15) it follows that, independently of the stimulating intensity, I_V always starts at zero value and then increases progressively to an (arbitrarily chosen) threshold value, I_{th} , during a time interval, Δt . Eq. (1a) can therefore be solved with the following boundary conditions

$$I_V(t=0) = 0, \quad (1c)$$

$$I_V(t=\Delta t) = I_{th}. \quad (1d)$$

As a result it was found that

$$\Delta t = \frac{1}{a} \ln \left[1 + \frac{a I_{th}}{b(I_{stim} - I_r)} \right], \quad (2)$$

which can be rewritten as

$$\Delta t = a_1 \ln \left[1 + \frac{b_1}{I_{stim} - I_r} \right], \quad (3)$$

where a_1 and b_1 are constants.

Hence the impulse frequency, f , of the encoder can be expressed as

$$f = \frac{1}{a_1 \ln \left[1 + \frac{b_1}{I_{stim} - I_r} \right] + t_{ap}} \quad (4)$$

where t_{ap} is the duration of the action potential proper.

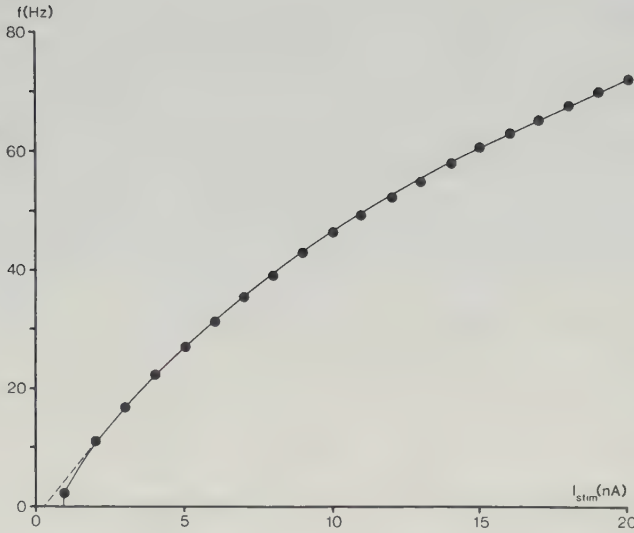


Fig. 16. Relationships between firing frequency, f , and stimulating current, I_{stim} , for the slowly adapting stretch receptor model (●). The solid line is drawn according to eq. (4), and the interrupted line (which differs from the solid line only at low stimulations) is computed from Eq. (6).

A graphic representation of Eq. (4) is given by the solid line in Fig. 16, where the points correspond to the frequency response of the model of the slowly adapting receptor neurone. It is seen that a close fit between the predictions of the receptor model and Eq. (4) is obtained by assuming that $a_1 = 97 \text{ ms}$, $b_1 = 1.5 \text{ nA}$, $I_r = 0.95 \text{ nA}$, and $t_{ap} = 6.5 \text{ ms}$.

Since b_1 may become small in relation to I_{stim} , Eq. (3) can be approximated to

$$\Delta t = \frac{a_1 b_1}{I_{stim} - I_r} = \frac{q_d}{I_{stim} - I_r}, \quad (5)$$

whence

$$f = \frac{1}{\frac{q_d}{I_{stim} - I_r} + t_{ap}} \quad (6)$$

In Fig. 16 it is shown that the prediction of Eq. (6) is identical with that of Eq. (4), except at low stimulation intensities (interrupted line), if it is assumed that $q_d=145$ pC, $I_r=0.3$ nA, and $t_{ap}=6.5$ ms. From an analysis of the equation it appears that I_r represents a bias current value shifting the stimulus-frequency relationship along the current scale, while q_d together with t_{ap} is a direct measure of the encoder sensitivity according to its exact definition. Despite its shortcomings at low stimulation and frequency values Eq. (6) will because of its simplicity and interpretability (see below) be used as basis for the further analysis.

Eq. (6) can in accordance with Eq. (1b) be rewritten as

$$f = \frac{1}{\frac{q_d}{I_d} + t_{ap}} \quad (7)$$

which, in physical terms, means that a certain (and constant) amount of charge, q_d , has, at all frequencies, to be displaced from the membrane capacitor by a driving current, I_d , during the interspike discharge phase. With a charge displacement of 145 pC/cell and a membrane capacitance of 13 $\mu\text{F}/\text{cm}^2$ the voltage change during the interspike discharge phase amounts to 19 mV, which is of an order as seen in the experiments or in model simulations (Fig. 14).

In distinction from the concept of Skaugen (1975), who treats the subthreshold membrane as a leaky capacitor, it is assumed in the above interpretation that the membrane corresponds to a capacitor short-circuiting a constant current generator. This view, which is supported by the fact that the cell's input resistance is very high during the interspike discharge phase (Fig. 2), implies that the duration of this phase is mainly determined by the strength of a more or less constant driving current and the value of the membrane capacitance. In agreement with this view it is, by model simulation, also found that the firing sensitivity is much more affected by variations of the membrane capacitance than by variation of the leak conductance, whose principal effect is to give rise to changes in the rheobase current (Fig. 17).

In conclusion it may be stated that despite its simplicity and strictly linear features the above formulations give an amazingly correct

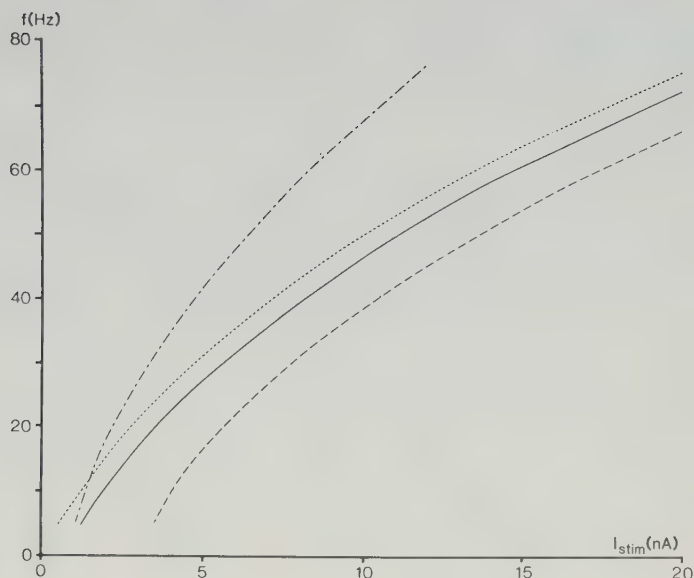


Fig. 17. Relationships between impulse frequency, f , and stimulating current, I_{stim} , for the slowly adapting receptor model in various experimental conditions. Control conditions (—); after doubling (---), and halving (-----) the resting leak conductance (g_L) ΔV_L ; after halving the membrane capacitance, C_m (-.-.-).

description of the encoder sensitivity which, after all, is determined by interaction of a number of highly non-linear membrane mechanisms. From a functional point of view such a "linearization" may be advantageous and it is therefore not surprising that its presence can be demonstrated also in other neuronal systems.

Application of the simplified frequency model to mammalian central neurones. In both motoneurones (Kernell & Sjöholm 1972, 1973) and dorsal spinocerebellar tract (DSCT) neurones of the cat (Gustafsson et al. 1978) the stimulation - frequency curve has a multisegmental shape. It may be subdivided into a primary range, in which the firing frequency increases slowly and more or less linearly with stimulation intensity, a secondary range of more steeply, and a tertiary range of, again, more slowly increasing stimulus dependent firing frequency. An example of this kind of relationship is, for a DSCT neurone, given by the points in the main graph in Fig. 18. As an explanation of the low stimulus sensitivity in the primary range it has been suggested that a K^+ current is quickly activated during the action potential, and slowly de-activated again during the

subsequent interspike discharge phase. As long as the stimulating current does not exceed the maximum value of the spike activated K^+ current, the firing frequency will be determined by the relaxation time of this current. This may be seen as if the spike activated K^+ current is putting a drain on the driving current source which, consequently, will have to accomplish a relatively large charge displacement in order to discharge the membrane capacitor to threshold levels. In Fig. 19 a fair reproduction of the primary range sensitivity was obtained by estimating q_d at about 40 pC (see inset), if I_r and t_{ap} were assumed to be 0.8 nA, and 0.6 ms, respectively.

As soon as the stimulating current exceeds the maximum value of the spike activated K^+ current, the latter loses its sensitivity controlling capability. This may be seen as a reduced drain on the driving current source which, now, can accomplish threshold depolarization with a lesser charge displacement. In Fig. 18 this effect is accounted for by letting q_d

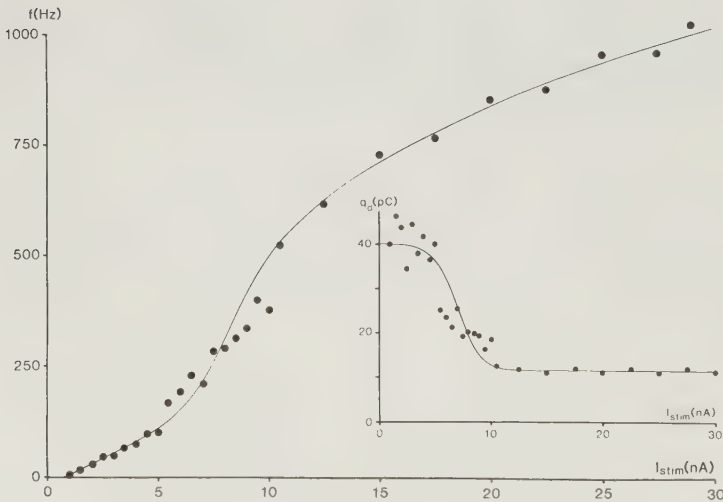


Fig. 18. Relationships between frequency of the first two impulses, f , and stimulating current, I_{stim} , for a dorsal spinocerebellar tract neurone (points after Gustafsson et al. 1978). The solid line is drawn in accordance with Eq. (6) using q_d values given by the continuous line in the inset. The points in the inset were derived from the points in the graph by assuming an action potential duration of 0.6 ms and a rheobase of 0.8 nA, while the smooth sigmoidal curve was computed in accordance with Eq. (A10).

fall with increasing stimulating current to a new constant value of about 10 pC. In the main graph it is shown that with this adjustment it is possible to achieve a fair reproduction of the cell's stimulus sensitivity in its secondary and tertiary ranges.

From the above example it appears that an essentially linear sensitivity adjustment exists in, for example, mammalian central neurones, even though conditions may be complicated by the fact that the activation of special current drains causes the sensitivity parameter, q_d , to shift between different (stable) values.

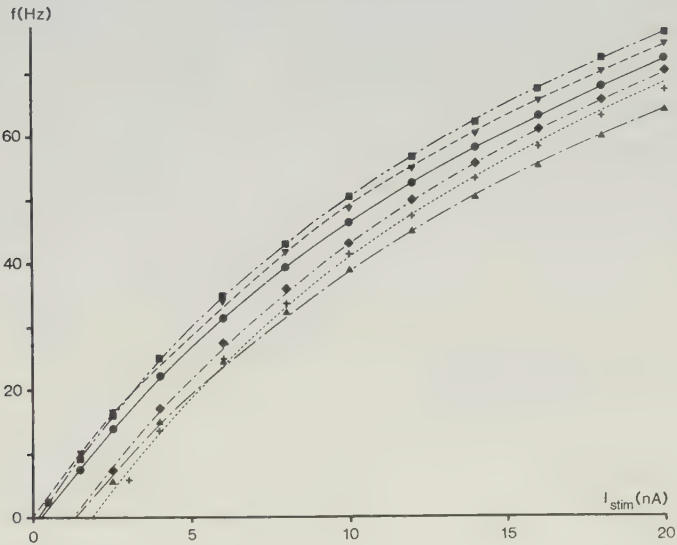


Fig. 19. Relationships between firing frequency, f , and stimulating current, I_{stim} , for the slowly adapting receptor model in various states of adaptation. Resting conditions (●); after setting l to 0.4 (▲); after setting r to 0.7 (■); after adjusting $[Na^+]_i$ to 30 mM (◆); after adjusting $[K^+]_i$ to 150 mM (▼); adapted conditions (+), which approximately correspond to the state of adaptation 20 s after the application of a 5 nA stimulating current. The different lines are computed from Eq. (6) using q_d and I_r values given in Table 2.

Effects of adaptation on the stimulus - frequency relationship

For an investigation of the effects of adaptation on the stimulus - frequency relationship, simulations of this relationship were performed with the parameters of the various adaptive systems (slow Na and K inactivations, and intracellular ion concentrations) set to different "adapted" values (Fig. 19, points). After this, predictions of Eq. (6) were fitted to these relationships by choosing the proper values of q_d and I_r while assuming that the action potential duration, t_{ap} , was not markedly affected by adaptation, and thus, could be maintained at its previous value of 6.5 ms. The results of this procedure are represented by the various curves in Fig. 19, which were computed using the values listed in Table 2. It is seen that by turning on the slow Na inactivation (decreasing l) there is an increase in q_d and I_r indicating that this system lowers firing sensitivity and raises the rheobase. A turning on of the slow K inactivation, on the other hand, has only little influence on the rheobase, but increases the firing sensitivity. The two slowly inactivating systems can therefore not be looked upon as being the exact functional opposites of each other. Concerning the effects of alterations in intracellular ion

Table 2. Values of q_d and I_r for different states of adaptation in the slowly adapting model

State of adaptation	q_d (pC)	I_r (nA)
control*	145	0.3
$l = 0.4$	167	1.3
$r = 0.7$	130	0.2
$[Na^+]_i = 30$ mM	145	1.3
$[K^+]_i = 150$ mM	138	0.0
adapted**	145	1.9

* control values $l = 0.84$, $r = 0.90$, $[Na^+]_i = 20$ mM, $[K^+]_i = 160$ mM

** adapted values $l = 0.40$, $r = 0.70$, $[Na^+]_i = 30$ mM, $[K^+]_i = 150$ mM

concentrations it appears that an increase in Na^+ leads to an elevation of the rheobase, through activation of the counter-excitatory pump current, while the firing sensitivity is kept constant. In contrast, an equally large decrease in K^+ slightly lowers the rheobase and somewhat increases the stimulus sensitivity. Finally, it is as an important point seen that with all the adaptive systems operating simultaneously (which approximately correspond to the state of adaptation 20 s after the application of a 5 nA stimulating current) the stimulus sensitivity is virtually unaffected, although there is a marked increase of the rheobase value.

Thus, in the present preparation the decrease in firing frequency during adaptation may be seen as a loss in stimulating current which has no effect on the cell's ability to rapidly respond to dynamic stimulus variations. Principally, this conclusion applies to both slowly and rapidly adapting cells whose firing properties are, despite apparently large differences, determined by very similar control mechanisms.

APPENDIX

THE RECEPTOR MODEL

1. Mathematical formulations

Membrane model

$$\frac{dV}{dt} = -\frac{1}{AC_m} [I_{\text{Na}} + (I_L)_{\text{Na}} + (I_p)_{\text{Na}} + I_K + (I_L)_K + (I_p)_K + I_{\text{inj}}] \quad (\text{A1})$$

Passive currents

$$I_{\text{Na}} = \bar{A} \bar{P}_{\text{Na}} m^2 h l \frac{VF^2}{RT} \frac{[\text{Na}^+]_o - [\text{Na}^+]_i \exp(VF/RT)}{1 - \exp(VF/RT)} \quad (\text{A2})$$

$$(I_L)_{\text{Na}} = A(P_L)_{\text{Na}} \frac{VF^2}{RT} \frac{[\text{Na}^+]_o - [\text{Na}^+]_i \exp(VF/RT)}{1 - \exp(VF/RT)} \quad (\text{A3})$$

$$I_K = A \bar{P}_K n^2 r \frac{VF^2}{RT} \frac{[K^+]_o - [K^+]_i \exp(VF/RT)}{1 - \exp(VF/RT)} \quad (A4)$$

$$(I_L)_K = A(P_L)_K \frac{VF^2}{RT} \frac{[K^+]_o - [K^+]_i \exp(VF/RT)}{1 - \exp(VF/RT)} \quad (A5)$$

$$(I_L)_{Cl} = A(P_L)_{Cl} \frac{VF^2}{RT} \frac{[Cl^-]_o - [Cl^-]_i \exp(-VF/RT)}{1 - \exp(-VF/RT)} \quad (A6)$$

Active currents

$$(I_p)_{Na} = AF \frac{(\bar{J}_p)_{Na}}{1 + \left[\frac{K_m}{[Na^+]_i} \right]^3} \quad (A7)$$

$$(I_p)_K = -(I_p)_{Na} / R_p \quad (A8)$$

Channel gating

$$\frac{dp}{dt} = (p_\infty - p) / \tau_p \quad (A9)$$

$$p_\infty = v_p + \frac{1 - v_p}{1 + \exp \left[\frac{z_p e}{kT} (V - V_p) \right]} \quad (A10)$$

$$\tau_p = \frac{Q_p \bar{\tau}_p}{\exp \left[\frac{\alpha_p z_p e}{kT} (V - V_p) \right] + \exp \left[\frac{(\alpha_p - 1) z_p e}{kT} (V - V_p) \right]} \quad (A11)$$

$$q_p = \left[\frac{1 - \alpha_p}{\alpha_p} \right]^{\alpha_p} + \left[\frac{1 - \alpha_p}{\alpha_p} \right]^{\alpha_p - 1} \quad (A12)$$

where p refers to any of the gating variables m, h, l, n, or r.

Ion fluxes and intracellular concentrations

$$\frac{d[Na^+]_i}{dt} = -\frac{K_r}{AF} [I_{Na} + (I_L)_{Na} + (I_p)_{Na}] \quad (A13)$$

$$\frac{d[K^+]_i}{dt} = -\frac{K_r}{AF} [I_K + (I_L)_K + (I_L)_{Cl} + I_{inj}]^* \quad (A14)$$

* Under normal conditions the intracellular K^+ concentration can be obtained without integration since the assumption of a constant intracellular Cl^- concentration implies that the decrease in intracellular K^+ must equal the calculated increase in intracellular Na^+ . This observation indicates that the right hand side of eq. (A14) must be the sum of all ion currents that are not Na^+ currents. The inclusion of $(I_L)_{Cl}$ and I_{inj} as pure K^+ currents is in this context equivalent to assuming that the intracellular Cl^- concentration is kept constant by an electro-neutral K-Cl transport system. Such systems have been found to exist in for example apical membranes of oxyntic cells (Wolosin & Forte 1981).

System of equations solved when adjusting the model to resting conditions

The parameters obtained in the initiation are the individual leak permeabilities $(P_L)_{Na}$, $(P_L)_K$, $(P_K)_{Cl}$, and the pump constant $K_m \cdot (P_r)_{Cl,K}$, $(g_L)_{\Delta V_L}$, and ΔV_L are leak determining parameters which only enter the model during the balancing procedure, where their information is transferred into the individual leak permeabilities.

$$I_{Na} + (I_L)_{Na} + (I_p)_{Na} + I_K + (I_L)_K + (I_p)_K + (I_L)_{Cl} = 0, \quad (A15)$$

$$I_{Na} + (I_L)_{Na} + (I_p)_{Na} = 0, \quad (A16)$$

$$(P_L)_{Cl} - (P_r)_{Cl,K} \cdot (P_L)_K = 0, \quad (A17)$$

$$I_L(V_{res} + \Delta V_L) - I_L(V_{res}) - A \cdot \Delta V_L \cdot (g_L)_{\Delta V_L} = 0. \quad (A18)$$

2. Explanation of symbols

A	projected membrane area
C_m	membrane capacitance per unit area
F	Faraday's constant
I_{inj}	through the KCl electrode experimentally injected current
I_K	gated K^+ current
I_L	total leak current
$(I_L)_{Cl}$	Cl^- leak component
$(I_L)_K$	K^+ leak component
$(I_L)_{Na}$	Na^+ leak component
I_{Na}	gated Na^+ current
$(I_p)_K$	K^+ fraction of pump current
$(I_p)_{Na}$	Na^+ fraction of pump current
$(J_p)_{Na}$	active Na^+ extrusion
$(\bar{J}_p)_{Na}$	maximum active Na^+ extrusion
$[K^+]_i$	intracellular K^+ concentration
$[K^+]_o$	extracellular K^+ concentration
K_m	the $[Na^+]_i$ value at which $(J_p)_{Na} = 0.5(\bar{J}_p)_{Na}$
K_r	ratio between projected membrane area (A) and cell volume (Vol)
$[Na^+]_i$	intracellular Na^+ concentration
$[Na^+]_o$	extracellular Na^+ concentration
$\bar{P}_K$	maximum permeability of gated K channel
$(P_L)_{Cl}$	Cl^- leak permeability
$(P_L)_K$	K^+ leak permeability
$(P_L)_{Na}$	Na^+ leak permeability
$\bar{P}_{Na}$	maximum permeability of gated Na channel
$(P_r)_{Cl,K}$	ratio between $(P_L)_{Cl}$ and $(P_L)_K$
Q_p	asymmetry factor

R	the universal gas constant
R_p	Na-K pumping ratio
T	absolute temperature
V	membrane voltage (inside minus outside)
Vol	cell volume
V_p	membrane voltage at which half of the gating particles, p , are in the "closed" state
V_{res}	resting membrane voltage
e	the electron charge
$(g_L)_{\Delta V_L}$	total leak conductance over the voltage drop ΔV_L
h	gating variable (rapid Na channel inactivation)
k	Boltzmann's constant
l	gating variable (slow Na channel inactivation)
m	gating variable (Na channel activation)
n	gating variable (K channel activation)
p	any of the gating variables m , h , l , n , or r
p_∞	stationary value of p
r	gating variable (slow K channel inactivation)
z_p	effective valency of the gating particles p
α_p	asymmetry parameter pertaining to p
ΔV_L	voltage drop over which the total leak conductance was estimated
v_p	minimum value of p
τ_p	relaxation time of p
$\bar{\tau}_p$	maximum relaxation time of p

3. Parameter values

Parameter	Value of parameter in slowly adapting model	Value of parameter in rapidly adapting model if different from value in slowly adapting model	Unit
A	$6 \cdot 10^4$		μm^2
$[\text{Cl}^-]_i$	46		mM
$[\text{Cl}^-]_o$	414		mM
C_m	13		$\mu\text{F}/\text{cm}^2$
$(\bar{J}_p)_{\text{Na}}$	$1 \cdot 10^{-9}$	$0.25 \cdot 10^{-9}$	$\text{mol}/\text{cm}^2 \text{ s}$
$[\text{K}^+]_i$ initial	160		mM
$[\text{K}^+]_o$	5		mM
$[\text{Na}^+]_i$ initial	20		mM
$[\text{Na}^+]_o$	330		mM
$\bar{P}_K$	$2 \cdot 10^{-4}$	$4 \cdot 10^{-4}$	cm/s
$\bar{P}_{\text{Na}}$	$6 \cdot 10^{-4}$	$10 \cdot 10^{-4}$	cm/s
$(P_r)_{\text{Cl},K}$	0.3		
R_p	1.5		
Vol	$2.5 \cdot 10^5$	$1.0 \cdot 10^5$	μm^3
V_h	-35		mV
V_l	-53		mV
V_m	-19	-13	mV
V_n	-18		mV
V_r	-56	-61	mV
V_{res}	-65		mV
$(g_L)_{\Delta V_L}$	0.35	0.5	mS/cm^2
z_h	-4.0		
z_l	-3.5		
z_m	3.1		
z_n	2.6		

Parameter	Value of parameter in slowly adapting model	Value of parameter in rapidly adapting model if different from value in slowly adapting model	Unit
z_r	-4.0		
α_h	0.5		
α_l	0.3		
α_m	0.3		
α_n	0.3		
α_r	0.2	0.4	
ΔV_L	-5	+5	mV
v_h	0		
v_l	0		
v_m	0		
v_n	0.03		
v_r	0.5	0.3	
$\bar{\tau}_h$	5		ms
$\bar{\tau}_l$	1700		ms
$\bar{\tau}_m$	0.3		ms
$\bar{\tau}_n$	6		ms
$\bar{\tau}_r$	2000	1000	ms

SUMMARY

1. The present study aims at the formulation of a comprehensive theory explaining the firing control exerted by the encoder mechanism of both slowly and rapidly adapting stretch receptor neurones of the lobster. As basic hypothesis of the investigation it was assumed that the control of impulse firing in relation to stimulation intensity (stimulus sensitivity) and time (frequency adaptation) is achieved by a special balance of membrane currents in sub- and near-threshold voltage regions.
2. The experimental part of the study includes (a) a characterization of different membrane properties by means of conventional physiological and pharmacological techniques including intracellular microelectrode registration of membrane voltage and currents, (b) measurements of intracellular ion concentrations and (c) an electronmicroscopical investigation of certain geometrical properties of the receptors.
3. In both neurones the following membrane currents were identified: (a) a tetrodotoxin sensitive Na^+ current, (b) a tetraethylammonium and 4-aminopyridine sensitive K^+ current, (c) a ouabain sensitive pump current, and (d) a remaining leak current carried by Na^+ , K^+ , and Cl^- in combination. Kinetically, these currents were characterized with respect to their voltage, time, and ion concentration dependence. In particular, it could be concluded that, in addition to obeying regular Hodgkin-Huxley kinetics, the Na^+ and K^+ currents are also subject to slow inactivations, and that the pump current is activated in response to intracellular Na^+ accumulation. From the measurements a mathematical receptor model was inferred employing constant field and state transition theories for description of the passive, and the continuity principle and saturation kinetics for description of the active membrane currents.
4. From the likeness between the firing control of the inferred mathematical model and the living receptors it is concluded that adaptation is caused by the above mentioned slow mechanisms, and that the different types of firing control displayed by the two receptor types can be referred to quantitative differences in some of the control

parameters whose influence on the spike generating mechanism decides whether the process of adaptation systematically will lead to a complete termination of the impulse activity, or only to a frequency reduction.

5. In a closer analysis of the mathematical models it is proposed that adaptation can be separated into two distinct effects corresponding to changes in stimulus sensitivity (gain) and stimulus intensity (rheobase adjustment), respectively. It is furthermore shown that in the present preparation adaptation is equivalent to a decrease in stimulus intensity. The analysis also predicts that stimulus sensitivity, firing adaptation, and termination of impulse firing are three rather independent encoder qualities that can be individually adjusted in order to meet the particular needs of a neurone.

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